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"BIOINSPIRED SYNTHESIS AND CHARACTERIZATION OF METAL OXIDE NANOPARTICLES FROM GARCINIA SPECIES AND EVALUATION OF ANTICANCER ACTIVITY"

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ABBREVIATIONS

- **NPs -** Nanoparticles
- **GC -** *Garcinia cambogia*
- **GI-** *Garcinia indica*
- **CuO -** Copper oxide
- **ZnO -** Zinc oxide
- **Gc-ZnO NPs -** *Garcinia cambogia*-zinc oxide nanoparticles
- **Gi-ZnO NPs -** *Garcinia indica*-zinc oxide nanoparticles
- **Gc-CuO NPs**- *Garcinia cambogia*-copper oxide nanoparticles
- **Gi-CuO NPs -** *Garcinia indica*-copper oxide nanoparticles
- **UV-vis spec -** Ultraviolet Visible Spectrophotometer
- **FT-IR -** Fourier Transform Infrared Spectroscopy
- **XRD -** X-Ray Diffraction
- **SEM -** Scanning Electron Microscopy
- **EDAX -** Energy Dispersive X-Ray Analysis
- **HR-TEM -** High Resolution Transmission Electron Microscopy
- **SAED -** Selected Area Electron Diffraction Pattern
- **DLS** - Dynamic Light Scattering
- **FRAP -** Ferric Ion Reducing Antioxidant Power
- **PM -** Phosphomolybdenum
- **DPPH -** 2, 2-Diphenyl-1-picrylhydrazyl
- **HEK 293 -** Human Embryonic Kidney Cells
- **A498 -** Human Renal Carcinoma Cells
- **RCC -** Renal Carcinoma Cells
- **Hrs -** Hours
- **MTT**- (3- (4,5-Dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide)
- **XTT**- (2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5carboxanilide)
- **DAPI -** 4’,6-Diamidino-2-phenylindole
- **FITC** - Fluorescein-5-isothiocyanate

CHAPTER I - INTRODUCTION

Cancer holds the top second cause when it comes to deadly disease of today's human population in entire Europe (1, 2). Among the 10 common death-causing cancers, kidney cancer is one of the debilitating health problems worldwide bannering renal cell carcinoma (RCC) as the most commonly known renal malignancy (3, 4). Couple of years ago, 1.8 crore (18 million) new cancer cases were diagnosed globally. RCC constituted around 2% of them; although it is relatively rare compared to other cancers. Both incidence and mortality are raising in an alarming rate of 3% per decade. The diagnosing techniques for renal cancer have advanced in different methods over the past decades. Surgery is the best therapy for early-stage kidney cancer, but survival rate was hardly 1 year. Specific therapies prevailed for advanced kidney cancer in the clinic since the disease is unsusceptible to chemotherapy (5). Researchers all over the world are effectively working on exploring a cost-effective, eco-friendly anticancer drug with little or no side effects. In the last fifteen years, more than 1,100 anticancer drugs were developed. Out of which, most of them are in clinical trials whereas only few drugs are in favorable features to get the approval from Food and Drug Administration (1). In this regard, the development of nanoparticles (NPs) based drugs have been effective in having minimal side effects and targeted action on cancer cells due to their large surface area. Especially inorganic NPs, including metal and metal oxides (gold, nickel, silver, iron oxide, zinc oxide, gadolinium, and titanium dioxide particles), are promising materials for their applications in medicines, such as cell imaging, bio sensing, drug/gene delivery, and cancer therapy (6).

Metal-based nanoparticles are called 'a wonder of modern medicine' and have sparked a lot of interest in the field of nanomedicine. Having high fraction of atoms, Metal oxide nano particles have been synthesized employing physical and chemical approaches which is hazardous, expensive and non-environment friendly (7). Hence, green synthesis of NPs was employed as an alternative way for easy, cost-effective, less toxic, facile synthesis of nano-scale materials. Metal oxide NPs are well-known for their efficacy in causing minimal side effects, selective targeting capability, high solubility and having a targeted effect on cancer cells (8). Nanoparticle production encompasses microwave or autoclave aided synthesis, microbe mediated synthesis, chemical synthesis, and physical techniques, however toxic chemicals limit their applicability and makes it uneconomical (9).

Metal oxides represent a family of materials that have a wide range of properties with potential applications. Among various metal oxides, zinc oxide (ZnO) and copper oxide (CuO) have been considered in the present work. Zinc oxide is an inorganic compound naturally occurring in crystalline structure. It is a multifunctional semiconducting metal oxide (10). Copper oxide (II) or cupric oxide is one of the inorganic compounds with the formula CuO (11). The application of ZnO and CuO NPs in many sectors and technologies such as biomedicine, electronics, environmental protection, ceramics, and rubber processing garnered significant scientific consideration (12).

According to Selim *et al.*, plant species are a good source for synthesizing nanoparticles on a wide scale since their production rate is quicker than that of other organisms (13). Furthermore, plant-derived nanoparticles contain bioactive components such as alkaloids, polyphenols, flavonoids, terpenoids, and others that aid in the bio-reduction, production, and stability of nanoparticles. And also varies in different shapes and sizes compared to other organisms such as bacteria, fungi, and algae.

Since ancient times, natural compounds are used as a source in developing new drugs (14). Cancer, diabetes, rheumatoid arthritis, stroke and coronary heart diseases are some of the fatal diseases, which are generally caused by ROS such as hydroxyl radical, super oxide anion and other exogenous factors. However, medicinal plants possessing antioxidant activity could be a potential lead for curing the above-mentioned diseases (15). Research into medicinal plants also provides essential knowledge about nutraceutical and herbal medicines due to enriched amount of secondary metabolites produced by them. Among many nutraceutical plants, Garcinia species are known to possess nutraceutical properties and proved to be rich sources of compounds with relevant therapeutical properties (16). Garcinia species are evergreen polygamous trees and shrubs covering a total of 400 species which covers both the hemispheres of tropical forests. Seventeen out of thirty-five Garcinia species are reported from Western Ghats, India (17). Among which, *Garcinia cambogia* (GC) and *Garcinia indica* (GI) are widely seen in the southern parts of Western Ghats, and are natively marketed as *Kodampuli* or *Gummi Gutta* and *Punarpuli* or *Kokum*, respectively.

In Ayurveda, it is said that the sour flavors, like those from Garcinia, activate digestion. This species has been used historically in India to support the treatment of wide-ranging health conditions (18). Garcinia species are grown for their fruits in Southeast Asia, coastal Karnataka, Kerala, West and Central Africa. These trees can grow on plain lands, on hilltops, but they are nurtured the best in the dry or rarely water-logged area and flooded

soils in river banks and valleys (19-21). Traditionally, Malabar tamarind and *kokum* are employed in herbal medicines to treat diarrhea, inflammatory ailments, dermatitis, bowel problems, and rheumatic pains and to forestall hyper perspiration. Fruits are used as anti-helminthic and cardio tonic (22, 23).

AIM

Aim of the present work was focused on optimization, green synthesis, and characterization of zinc oxide and copper oxide nanoparticles and to evaluate their antioxidant and anticancer activity of selected Garcinia species.

OBJECTIVES

1. Collection, identification, and authentication of *Garcinia cambogia* and *Garcinia indica* leaves.
2. Preparation of aqueous leaf extracts of *Garcinia cambogia* and *Garcinia indica* and evaluation of preliminary phytochemical screening and GC-MS analysis.
3. Optimization, green synthesis and characterization of zinc oxide and copper oxide nanoparticles from *Garcinia cambogia* leaves.
4. Optimization, green synthesis and characterization of zinc oxide and copper oxide nanoparticles from *Garcinia indica* leaves.
5. Evaluation of antioxidant activity of the green synthesized zinc oxide and copper oxide nanoparticles of *Garcinia cambogia* and *Garcinia indica* leaves.
6. Evaluation of cytotoxicity activity of the green synthesized zinc oxide and copper oxide nanoparticles of *Garcinia cambogia* and *Garcinia indica* leaves.
7. Evaluation of apoptosis activity of the green synthesized zinc oxide and copper oxide nanoparticles of *Garcinia cambogia* and *Garcinia indica* leaves.

CHAPTER II - REVIEW OF LITERATURE

The recently published literature reports relevant to the present study is discussed in the chapter. This chapter also deals with the information given in the introduction providing the background of green nanotechnology and experimental procedures for the synthesis of ZnO and CuO nanoparticles through green routes and the techniques used for their characterizations. The literature survey on an antioxidant and anticancer activity from different plants and their importance in the field of nanotechnology has also been discussed.

CHAPTER III - MATERIALS AND METHODS

This chapter is divided into four sections. Instruments and chemicals (Reagents and Solvents) used for this research work are given in part I. In part II the collection of plants, preparation of extracts, preliminary phytochemical screening, GC-MS analysis, optimization and synthesis of metal oxide nanoparticles (ZnO NPs and CuO NPs) were discussed. Part III has the demonstration the instrumental techniques used for characterization of synthesized nanoparticles. The nanomaterials obtained were characterized using the techniques like UV-Visible Spectrophotometer (UV-vis spec), Fourier Transformed Infrared (FT-IR), Spectroscopy, X-ray Diffraction Analysis (XRD), Scanning Electron Microscope (SEM), Energy dispersive X-ray Analysis (EDAX), High Resolution of Transmission Electron Microscope (HR-TEM), Selected Area Electron Diffraction (SAED), Dynamic Light Scattering (DLS) and particle size analyzer are given. Part IV deals with the assay procedures of antioxidant and anticancer activities of GC and GI leaf extracts along with their respective metal oxide nanoparticles (ZnO and CuO NPs). Statistical analysis was carried out wherever necessary. All the values were expressed as mean ± SD (n=3).

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The leaves of *G. cambogia* and *G. indica* were collected from the Central Horticultural Experiment Station, Chettalli, Kodagu district of Karnataka, India, situated at latitude 12.3890˚ and longitude 75.8453˚. Both species of Garcinia were identified and authenticated by Principal Scientist at PND Herbarium, Mangalore, Karnataka, India vide letter no. SKPND: CR: 113: Herbarium Collection/19-20. The herbarium is kept at PND

Herbarium, Mangalore as *Garcinia indica* (Thouars) Choisy (accession no. 2286) and *Garcinia gummi-gutta* (L.) Roxb. (Accession no. 9743) for further reference.

PHYTOCHEMICAL SCREENING

Qualitative phytochemical analysis

The aqueous extracts of *G. cambogia* and *G. indica* leaves were subjected to preliminary phytochemical screening following standard methods in order to find out the presence of following constituents (24-26).

Quantitative analysis

a. **Estimation of total phenolic content:** Estimation of Folin-Ciocalteu test was used to assess the total phenolic content of the aqueous extracts by following Noreen *et al*., (2017) with slight modifications using Gallic acid as the standard (27).
b. **Estimation of total flavonoid content:** Estimation of total flavonoid content of the aqueous extracts by aluminium colorimetric technique with slight modifications (28) using quercetin as the standard.
c. **Estimation of total alkaloid:** Determination of total alkaloid content in the extracts was determined by following standard protocol (29).
d. **Estimation of total saponin:** Determination of total saponin content in the extracts was determined by following standard protocol (29).

GC-MS ANALYSIS FOR PLANT EXTRACTS

GC-MS was performed at the Centre for Analytical Instrumentation Kerala, India. Identification was supported based on the molecular structure, molecular mass, and calculated fragments. Interpretation of GC-MS was conducted using standard mass spectral database of Wiley and National Institute Standard and Technology (NIST) libraries. The relative percentage amount of every component was calculated by comparing its average peak area to the entire area (30).

OPTIMIZATION OF METAL OXIDE NANOPARTICLES

The physicochemical factors affecting metal oxide nanoparticle synthesis was studied by optimizing the biomass concentration, metal ion concentration and temperature for green synthesis of zinc oxide and copper oxide nanoparticles. These parameters are highly

influential in order to maintain the shape, size, morphology, stability, and enhance the product yield (31).

SYNTHESIS OF ZnO AND CuO NPs FROM GC AND GI LEAF EXTRACTS

Metal oxides like zinc and copper oxide nanoparticles were synthesized using zinc nitrate hexahydrate and cupric nitrate trihydrate respectively by following the previously described method (32) with slight modifications.

ANALYTICAL TECHNIQUES

Characterization of biosynthesized ZnO and CuO nanoparticles

- **UV- visible spectrophotometer (UV-vis spec):** UV-vis spectra were recorded using UV-Visible spectrophotometer (Shimadzu, UV-1800). It is a very useful analytical technique as it can be used to determine the amount of substance present in a sample. Double distilled water was used as the reference (blank) solution with matched pair of Teflon stoppered quartz cuvettes of path length 1 cm for the entire study.
- **Fourier Transformed Infrared Spectroscopy (FTIR):** FTIR spectra of all synthesized samples were recorded by Shimadzu. The dried sample is mixed well with KBr (1:100 time) then to get pellet form for FTIR analysis.
- **X-ray diffraction (XRD):** In the present work, crystal structure, phase and crystallite size of the synthesized nanoparticles were determined by XRD analysis. The X-ray diffraction was carried out by Xpert MPD Philips, Holland.
- **Scanning Electron Microscopy (SEM):** In this study, the morphology analyses were carried out using ZEISS scanning electron microscope.
- **Energy Dispersive X-Ray** (**EDAX):** EDAX analysis is a standard method for local identification of elements within a sample in a SEM. In this work, EDAX analysis was performed using ZEISS. This provided quantitative elemental compositions for individual nanostructures.
- **Transmission electron microscopy (TEM):** TEM is a unique tool for characterizing crystalline structure and microstructure of materials simultaneously by diffraction and imaging techniques. In this work, TEM analysis was performed using Jeol/JEX 2100.

- **Selected Area Electron Diffraction pattern:** The SAED pattern can give information on the crystallography and crystal orientation of a sample. In this work, SAED analysis was performed using Jeol/JEX 2100.
- **Particle size distribution analysis:** The size distribution or average size of the synthesized nanoparticles was determined by dynamic light scattering (DLS) method. The DLS technique uses light to determine the size of particles in a solution. A laser emits light at a certain frequency, which is then transmitted through the solution. The analysis was performed using Jeol/JEX 2100.
- **Zeta-potential measurements:** The synthesised nanoparticles were evaluated to their surface charge in SZ-100, Horiba Scientific.

***INVITRO* ANTIOXIDANT ACTIVITY**

Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells and biological targets to cause various diseases (33). Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. In the current research, antioxidant activity of GC and GI extracts along with their respective metal oxide nanoparticles (ZnO and CuO NPs) were determined using three different assays:

- 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay
- Ferric Ion Reducing Antioxidant Power (FRAP) assay
- Phosphomolybdenum (PM) assay

a) DPPH assay: Free radical scavenging effect of aqueous plant extracts of GC and GI leaves along with their respective metal oxide nanoparticles (ZnO and CuO NPs) in different concentrations, ranging from 12.5 µg/mL to 200 µg/mL was determined using the DPPH method described by Williams *et al*., (1995) with slight medications (34).

b) FRAP assay: FRAP assay was used to measure the total antioxidant power of the extracts. The FRAP assay of aqueous crude extracts of GC and GI along with their respective metal oxide nanoparticles (ZnO and CuO NPs) in different concentrations, ranging from 12.5 µg/mL to 200 µg/mL was performed according to Benzie and Strain (1996) with slight modifications (35).

c) PM assay: The total antioxidant activity of aqueous crude extracts of GC and GI along with their respective metal oxide nanoparticles (ZnO and CuO NPs) in different concentrations, ranging from 12.5 µg/mL to 200 µg/mL was estimated by PM assay using the standard procedure (Prieto *et al.,* 1999) with slight modifications (36).

INVITRO ANTICANCER ACTIVITY

a) Cytotoxicity study

The human renal carcinoma cells (A498) and Human embryonic kidney cells (HEK 293) were procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cytotoxic activity of the aqueous extracts of GC and GI leaves along with their metal oxide nanoparticles (ZnO and CuO NPs) was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) assay and XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) assay by following Sudha *et al.,* (2020) with slight modifications (37). The cells were treated with a different concentration range of GC and GI leaves along with their metal oxide nanoparticles (ZnO and CuO NPs), ranging from 6.25-200 µg/mL followed by incubation for 48 hrs respectively. Cisplatin was used as the standard drug to compare the effects induced for HEK293 human embryonic kidney cell and A498 human kidney cancer cell lines.

b) Selectivity index (SI)

The A498 and HEK293 cells were used to measure the SI. The SI, which represents the cytotoxic selectivity (*i.e.,* drug safety), for both plant extracts and their respective metal oxide nanoparticles was calculated using the formula.

c) 4, 6-Diamidino-2-phenylindole (DAPI)

DAPI staining was carried out according to the method described by Jordana *et al.,* (2021) with slight modifications (38). Cell nuclear morphology was evaluated by fluorescence microscopy following DAPI staining. The samples were treated with different concentration IC_{25}, IC_{50} and IC_{75} for 24 hrs.

d) Annexin V-FITC/PI

Annexin V-FITC (Fluorescein isothiocyanate) was performed to determine cell death mode by following the method of Amin *et al.,* (2017) with slight modifications (39). The samples were treated with different concentration (IC_{25}, IC_{50} and IC_{75}) for 12 hrs.

STATISTICAL ANALYSIS

The study was carried out in triplicates (n=3) and results were expressed as mean ± standard deviation. Statistical significance between groups was assessed using two-way ANOVA (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) as compared to standard group using Graph Pad Prism 5 software (Version 5.03).

CHAPTER IV - RESULTS AND DISCUSSION

Biosynthesis of zinc oxide and copper oxide nanoparticles using aqueous leaf extract of *Garcinia cambogia* and its biological activities

PHYTOCHEMICAL ANALYSIS

Qualitative analysis of aqueous extract of GC: The qualitative phytochemical analysis of *Garcinia cambogia* leaf aqueous extract revealed the presence of active constituents like phenolics, alkaloids, flavonoids, terpenoids, saponins, glycosides, cardiac glycosides, steroids, and absence of tannins and resins.

Quantitative analysis of aqueous extract of GC: GC extract showed highest flavonoids (2.35±0.49 QE/g extract mg/g) followed by saponin (1.323±0.025 mg/g), phenolic (1.05±0.49 GAE/g extract mg) compounds and alkaloids (0.243±0.002 mg/g).

GC-MS ANALYSIS

The presence of phytochemicals from aqueous leaf of *G. cambogia* was determined by GC-MS. The bioactive compounds were predicted by their retention time peak, area percentage (%), MW, and molecular formula with the aid of Wiley and NIST libraries. The GC-MS chromatogram of aqueous leaf of *G. cambogia* revealed three peaks.

OPTIMIZATION OF ZINC OXIDE NANOPARTICLES DERVIVED FROM *GARCINIA CAMBOGIA* (Gc-ZnO NPs)

Optimization of reaction conditions like the quantity of GC extract, precursor metal ion concentration and temperature were carried out using UV-vis spec.

- **Effect of plant extract concentration**: Gc-ZnO NPs were synthesized by introducing 2.5 mL, 5 mL, and 10 mL of GC plant extract. 5 mL of plant extract revealed a maximum peak at 266 nm with no broadening peak.
- **Effect of temperature**: The influence of temperature on the synthesis of Gc-ZnO NPs using GC extract was greatest at 400 °C in a 50 mM concentration.
- **Effect of precursor metal ion concentration:** The optimum concentration required by the UV-vis spec at the maximum wavelength of 266 nm was 50 mM, with peak sharpening.

OPTIMIZATION OF COPPER OXIDE NANOPARTICLES DERVIVED FROM *GARCINIA CAMBOGIA* (Gc-CuO NPS)

- **Effect of plant extract concentration**: Gc-CuO NPs were synthesized by introducing 2.5 mL, 5 mL and 10 mL of GC plant extract. 2.5 mL of plant extract revealed a maximum peak at 265 nm.
- **Effect of temperature**: The influence of temperature on the synthesis of Gc-CuO NPs using GC extract was greatest at 400 °C in a 100 mM concentration.
- **Effect of precursor metal ion concentration**: The optimum concentration required by the UV-vis spec at the maximum wavelength of 265 nm was 100 mM, with peak sharpening.

SYNTHESIS OF Gc-ZnO AND Gc-CuO NANOPARTICLES

Gc-ZnO NPs were synthesized according to the established protocols using the aqueous leaf extract from GC. After determining the above optimal conditions in each of the effective parameters, (50 mM of zinc nitrate, 5 mL of GC extract and calcination at 400 ℃ temperature), the GC NPs were synthesized and was characterized using various physico-chemical parameters to ascertain their size, shape, and functionalization (32).

Gc-CuO NPs were synthesized according to the established protocols using the aqueous leaf extract from GC. After determining the above optimal conditions in each of the effective parameters, (100 mM of copper nitrate, 2.5 mL of GC extract and calcination at 400 ℃ temperature), the Gc-CuO NPs were synthesized and was characterized using various physico-chemical parameters to ascertain their size, shape, and functionalization.

CHARACTERIZATION OF Gc-ZnO AND Gc-CuO NPs

UV-visible spectrophotometer: UV-visible spectra for ZnO nanoparticles, was observed at 266 nm and confirms the formation of ZnO and the absorption peak at 265 nm and revealed the formation of CuO.

FTIR analysis: FTIR analysis confirms the functional groups present in the photosynthesized CuO and ZnO nanoparticles. In FTIR spectra of metallic nanoparticles confirmed the presence of alcohols, polyphenols, alkenes, and presence of tertiary alcohol. This shows the special character of biological molecules that can act both as reducing and capping agent in the synthesis of zinc and copper oxide nanoparticles.

XRD: The hexagonal phase wurtzite structure for Gc-ZnO NPs were exhibited and the

average crystallite size of nanoparticles was found to be 11.3 nm respectively and the average crystallite size of Gc-CuO nanoparticles was found to be 19.39 nm respectively. The CuO nanoparticle showed the monoclinic phase.

SEM analysis: The SEM analysis revealed rod-like structure in Gc-ZnO NPs and in Gc-CuO NPs rod and spherical shape was observed with some agglomerations in the nanoparticles.

EDAX: The quantitative analysis of EDAX spectrum reflects the presence of Zn and O peaks and Cu and O Peaks clearly indicated that the product was of pure ZnO and CuO lacking of impurities.The other weaker signals of K, L and S are owing to use precursor salts for the synthesis of nanoparticles (40).

HR-TEM: The synthesised Gc-ZnO NPs have nanoscale homogeneity of 14-32 nm and are mostly hexagonal forms whereas the synthesised Gc-CuO NPs have nanoscale homogeneity of 31 nm and are mostly spherical.

SAED: SAED revealed the ring patterns appeared with the light spots on the dark field. It reflects the crystalline structure of the Gc-ZnO NPs and Gc-CuO NPs.

Zeta potential: Zeta potential provides the zeta value which shows surface charge and stability. The zeta Potential value of Gc-ZnO NPs is 57.9 mV and Gc-CuO NPs is -25.8 mV demonstrating its stability.

Particle size analysis: The average particle size of the GC-ZnO NPs using DLS method was found to be about 133.8 nm and 123.4 nm for Gc-CuO NPs, both biosynthesized sample exhibited the average particle size fairly higher than the X-ray diffraction method, which might be due to smaller nanoparticles agglomeration.

INVITRO **ANTIOXIDANT ACTIVITY OF METAL OXIDE NANOPARTICLES AND GC EXTRACT**

DPPH assay: GC extract showed significantly greater DPPH scavenging action at lower concentrations (12.5 to 50 µg/mL) compared to Gc-ZnO NPs, Gc-CuO NPs and ascorbic acid. Gc-ZnO NPs, on the other hand, demonstrated a highly significant result at lower concentrations along with GC extract compared to Gc-CuO NPs and ascorbic acid but Gc-ZnO NPs showed better result than GC extract as the concentration was increased. All the extract showed dose dependent inhibition as the concentration was increased

FRAP assay: Gc-CuO NP extract showed highly significantly greater FRAP action at all the concentrations compared to GC extract, Gc-ZnO NPs, and ascorbic acid. GC extract, on the other hand, demonstrated a significant result at lower concentrations (12.5 to 100

µg/mL) along with Gc-CuO NP extract compared to Gc-ZnO NPs and ascorbic acid. All the extract showed dose dependent inhibition as the concentration was increased.

PM assay: All extract showed significant PM action which are comparable to ascorbic acid. However, Gc-CuO NP extract demonstrated better PM activity compared to GC extract, Gc-ZnO NPs and ascorbic acid. All the extract showed dose dependent inhibition as the concentration was increased**.**

INVITRO **ANTICANCER ACTIVITY OF METAL OXIDE NANOPARTICLES AND GC EXTRACT**

Cytotoxicity studies of MTT assay for Gc-ZnO NPs, Gc-CuO NPs and GC extract

MTT assay (HEK293 cell line): At lower concentration (6.25 µg/mL), 90% viable cells were present in GC extract and Gc-ZnO NP extract while nearly 80% cells were unharmed in Gc-CuO NPs extract. However, as the concentration was increased (200 µg/mL), less than 50% living cells can be seen in metal oxide nanoparticles and almost 60% unharmed cells were present in GC extract.

MTT assay (A498 cell line): At lower concentration (6.25 µg/mL), metal oxide nanoparticles inhibited more than 60% cancer cells whereas GC extract inhibited very less amount of cancer cells. As the concentration was increased (200 µg/mL), metal oxide nanoparticles inhibited almost 90% of cancer cells in 48 hrs. These results suggest that the effect of GC extract, Gc-ZnO NP and Gc-CuO NP extract are dose dependent. Metal oxide nanoparticles are ideal choice at 6.25 µg/mL because no significant effect was observed in normal renal cells at this concentration. Lower the IC_{50} value, higher the anticancer activity. SI values > 2 was considered as high selectivity. Cisplatin was used as the standard drug.

Cytotoxicity studies of XTT assay for Gc-ZnO NPs, Gc-CuO NPs and GC extract

XTT assay (HEK293 cell line): At lower concentration (6.25 µg/mL), 90% viable cells were present in GC extract and Gc-ZnO NPs extract while nearly 70% cells were unharmed in Gc-CuO NPs extract. However, as the concentration was increased (200 µg/mL), almost 50% living cells can be seen in metal oxide nanoparticles and almost 70% unharmed cells were present in GC extract.

XTT assay (A498 cell line): At low concentration (25 µg/mL), metal nanoparticles

inhibited more than 75% cancer cells whereas GC extract showed good inhibition at the concentration range of 50 µg/mL. As the concentration was increased (200 µg/mL), metal oxide nanoparticles inhibited almost 90% of cancer cells in 48 hrs respectively. These results suggest that the effect of GC extract, ZnO NP and Gc-CuO NP extract are dose dependent. Based on the IC_{50} value and selectivity index, it is clear that metal oxide nanoparticles showed better cytotoxic effects compared to plant extract and cisplatin. Lower the IC_{50} value, higher the anticancer activity. SI values > 2 was considered as high selectivity. Cisplatin was used as the standard drug.

Apoptosis activity of metal oxide nanoparticles and GC extract

DAPI: DAPI staining revealed that all treated A498 cells visibly appeared to exhibit small nuclei with bright chromatin condensation, blebbing, nuclear fragmentation, apoptotic body formation, chromatin condensation, nuclear shrinkage and nuclear blebbing. However the control cells remained intact and evenly shaped. The DAPI staining noticeably showed apoptotic morphological changes in GC extract, Gc-ZnO NPs, and Gc-CuO NPs in terms of both nuclear condensation and cell structure loss.

Annexin V-FITC/PI: The percentage of viable, early apoptotic, late apoptotic, and necrotic cells of negative control (untreated), positive control (Cisplatin), GC extract, Gc-ZnO NPs, Gc-CuO NPs, and A498 cells were treated for 12 hrs., was determined by a flow cytometer. There was almost 100% of negative control A498 culture cells were viable, and no apoptosis occurred after 12 hrs of incubation. However, treated cells with GC extract, Gc-ZnO NPs, and Gc-CuO NPs, the viable cell percentage reduced significantly at IC_{25}, IC_{50} and IC_{75} respectively. Furthermore, the percentage of cells at apoptotic stage increased significantly at all concentrations compared to control group.

CHAPTER - V

Biosynthesis of zinc oxide and copper oxide nanoparticles using aqueous leaf extract of *Garcinia indica* and its biological activities

PHYTOCHEMICAL ANALYSIS

Qualitative phytochemical analysis of GI: The qualitative phytochemical analysis of GI leaf aqueous extract revealed the presence of active constituents like phenolics, alkaloids, flavonoids, terpenoids, saponins, glycosides, cardiac glycosides, and absence of steroids, tannins, and resins.

Quantitative analysis of aqueous extract of GI: GI contain highest phenolic compounds (3.0±0.47 mg GAE/g extract) followed by flavonoids (2.21±0.33 mg QE/g extract), saponin (1.423±0.030 mg/g) and alkaloids (0.059±0.003 mg/g).

GC-MS ANALYSIS

The presence of phytochemicals from aqueous leaf of *G. indica* was determined by GC-MS. The bioactive compounds were predicted by their retention time peak, area percentage (%), MW, and molecular formula with the aid of Wiley and NIST libraries. The GC-MS chromatogram of aqueous leaf of *G. cambogia* revealed five peaks.

OPTIMIZATION OF GI ZINC OXIDE NANOPARTICLES DERVIVED FROM *GARCINIA INDICA* (Gi-ZnO NPs)

Optimization of reaction conditions like the quantity of GI extract, precursor metal ion concentration and temperature were carried out using UV-vis spec.

- **Effect of plant extract concentration**: Gi-ZnO NPs were synthesized by introducing 2.5 mL, 5 mL, and 10 mL of GI plant extract. 10 mL of plant extract revealed a maximum peak at 276 nm.
- **Effect of temperature**: The influence of temperature on the synthesis of Gi-ZnO NPs using GI extract was greatest at 400 °C in a 75 mM concentration.
- **Effect of precursor metal ion concentration:** The optimum concentration required by the UV-vis spec at the maximum wavelength of 276 nm was 75 mM, with peak sharpening. As a result, 75 mM being optimum.

OPTIMIZATION OF COPPER OXIDE NANOPARTICLES DERVIVED FROM *GARCINIA INDICA* (Gi-CuO NPs)

- **Effect of plant extract concentration:** Gi-CuO NPs were synthesized by introducing 2.5 mL, 5 mL, and 10 mL of GI plant extract. 10 mL of plant extract revealed a maximum peak at 265 nm.
- **Effect of temperature**: The influence of temperature on the synthesis of Gi-CuO NPs using GI extract was greatest at 500 °C in a 100 mM concentration.
- **Effect of precursor metal ion concentration:** The optimum concentration required by the UV-vis spec at the maximum wavelength of 265 nm was 100 mM, by the spectra, with peak sharpening.

SYNTHESIS OF Gi- ZnO AND Gi-CuO NANOPARTICLES

The Gi-ZnO-NPs were synthesized according to the established protocols using the aqueous leaf extract from GI. After determining the above optimal conditions in each of the effective parameters, (75 mM of zinc nitrate, 10 mL of GI extract and calcination at 400 ℃ temperature), the Gi-ZnO NPs were synthesized and was characterized using various physico-chemical parameters to ascertain their size, shape, and functionalization (32).

The Gi-CuO-NPs were synthesized according to the established protocols using the aqueous leaf extract from GI. After determining the above optimal conditions in each of the effective parameters, (100 mM of copper nitrate, 10 mL of GI extract and calcination at 500 ℃ temperature), the Gi-CuO NPs were synthesized and was characterized using various physico-chemical parameters to ascertain their size, shape, and functionalization.

CHARACTERIZATION OF Gi-ZnO AND Gi-CuO NPs

UV-vis spectrophotometer: UV-vis spectra for ZnO nanoparticles, was observed at 276 nm and confirms the formation of ZnO and the absorption peak at 265 nm and revealed the formation of CuO.

FTIR analysis: FTIR analysis confirms the functional groups present in the photosynthesized CuO and ZnO nanoparticles. In FTIR spectra of metallic nanoparticles confirmed the presence of alcohols, polyphenols, alkenes, and presence of tertiary alcohol. This shows the special character of biological molecules that can act both as reducing and capping agent in the synthesis of zinc and copper oxide nanoparticles.

XRD analysis: The hexagonal phase wurtzite structure for Gi-ZnO NPs were exhibited

and the average crystallite size of nanoparticles was found to be 8.75 nm respectively and the average crystallite size of Gi-CuO nanoparticles was found to be 7.78 nm respectively. The CuO nanoparticle showed the monoclinic phase

SEM analysis: The SEM analysis revealed spherical shape in Gi-ZnO NPs and in Gi-CuO Nps in spherical shape was observed with some agglomerations in the nanoparticles,

EDAX: The quantitative analysis of EDAX spectrum reflects the presence of Zn and O peaks in and Cu and O Peaks in clearly indicated that the product was of pure ZnO and CuO lacking of impurities. The other weaker signals of K, L and S are owing to use precursor salts for the synthesis of nanoparticles (40).

HR-TEM: The synthesised Gi-ZnO NPs have nanoscale homogeneity of 10-20 nm and are mostly hexagonal forms whereas the synthesised Gi-CuO NPs have nanoscale homogeneity of 11-29 nm and are mostly spherical.

SAED: SAED revealed the ring patterns appeared with the light spots on the dark field. It reflects the crystalline structure of the Gi-ZnO NPs and Gi-CuO NPs.

Zeta potential: Zeta potential provides the zeta value which shows surface charge and stability. The zeta Potential value of Gi-ZnO NPs is -45.0 mV and Gi-CuO NPs is -26.7 mV demonstrating its stability.

Particle size analysis: The average particle size of the Gi-ZnO NPs using DLS method was found to be about 133.9 nm and 123.6 nm for Gi-CuO NPs, both biosynthesized sample exhibited the average particle size fairly higher than the X-ray diffraction method, which might be due to smaller nanoparticles agglomeration.

***INVITRO* ANTIOXIDANT ACTIVITY OF METAL OXIDE NANOPARTICLES AND GI EXTRACT**

DPPH assay: GI extract showed significantly greater DPPH scavenging action at lower concentrations (12.5 to 50 µg/mL) compared to Gi-ZnO NPs, Gi-CuO NPs and ascorbic acid. Gi-ZnO NPs, on the other hand, demonstrated a highly significant result at lower concentrations along with GI extract compared to Gi-CuO NPs and ascorbic acid but Gi-ZnO NPs showed better result than GI extract as the concentration was increased. All the extract showed dose dependent inhibition as the concentration was increased.

FRAP assay: All extract showed significantly greater FRAP action at all the concentrations compared to ascorbic acid. However, Gi-CuO NP extract demonstrated highly significant activity compared to GI extract, Gi-ZnO NPs and ascorbic acid. All the extract showed dose dependent inhibition as the concentration was increased.

PM assay: GI extract showed significantly greater PM action at lower concentrations (12.5 to 100 µg/mL) compared to Gi-ZnO NPs, Gi-CuO NPs and ascorbic acid. All the extract showed dose dependent inhibition as the concentration was increased.

INVITRO ANTICANCER ACTIVITY OF METAL OXIDE NANOPARTICLES AND GI EXTRACT

Cytotoxicity studies of MTT assay for Gi-ZnO NPs, Gi-CuO NPs and GI extract

MTT assay (HEK293 cell line): At lower concentration (6.25 µg/mL), 90% viable cells were present in GI extract while nearly 80% cells were unharmed in metal oxide nanoparticles. However, as the concentration was increased (200 µg/mL), less than 50% living cells can be seen in metal oxide nanoparticles and 60% unharmed cells were present in GI extract.

MTT assay (A498 cell line): At lower concentration (6.25 µg/mL), metal oxide nanoparticles inhibited more than 60% cancer cells whereas GI extract inhibited nearly 30% of cancer cells. As the concentration was increased (200 µg/mL), metal oxide nanoparticles killed almost 90% of cancer cells in 48 hrs respectively. These results suggest that the effect of GI extract, Gi-ZnO NP and Gi-CuO NP extract are dose dependent. Metal oxide nanoparticles are ideal choice at 6.25 µg/mL because no significant effect was observed in normal renal cells at this concentration. Lower the IC_{50} value, higher the anticancer activity. SI values > 2 was considered as high selectivity. Cisplatin was used as the standard drug.

Cytotoxicity studies of XTT assay for Gi-ZnO NPs, Gi-CuO NPs and GI extract

XTT assay (HEK293 cell line):t lower concentration (6.25 µg/mL), 90% viable cells were present in GI extract and Gi-ZnO NP extract while nearly 60% cells were unharmed in Gi-CuO NPs extract. However, as the concentration was increased (200 µg/mL), almost 50% living cells can be seen in metal oxide nanoparticles and 60% unharmed cells were present in GI extract.

XTT assay (A498 cell line): At lower concentration (12.5 µg/mL), Gi-CuO NPs inhibited more than 75% cancer cells whereas Gi-ZnO NPs showed 80% inhibition at the concentration range of 50 µg/mL. As the concentration was increased (200 µg/mL), metal

oxide nanoparticles inhibited almost 90% of cancer cells in 48 hrs respectively. These results suggest that the effect of GI extract and metal oxide nanoparticles are dose dependent. Based on the IC_{50} value and selectivity index, it is clear that metal nanoparticles showed better cytotoxic effects compared to plant extract and cisplatin. Lower the IC_{50} value, higher the anticancer activity. SI values > 2 was considered as high selectivity. Cisplatin was used as the standard drug.

Apoptosis activity of metal oxide nanoparticles and GI extract

DAPI: DAPI staining revealed that all treated A498 cells visibly appeared to exhibit small nuclei with bright chromatin condensation, blebbing, nuclear fragmentation, apoptotic body formation, chromatin condensation, nuclear shrinkage and nuclear blebbing. However the control cells remained intact and evenly shaped. The control cells, however, remained intact and evenly shaped.The DAPI staining noticeably showed apoptotic morphological changes in GI extract, Gi-ZnO NPs, and Gi-CuO NPs in terms of both nuclear condensation and cell structure loss.

Annexin V-FITC/PI: The percentage of viable, early apoptotic, late apoptotic, and necrotic cells of negative control (untreated), positive control (Cisplatin), GI extract, Gi-ZnO NPs, Gi-CuO NPs, and A498 cells were treated for 12 hrs was determined by a flow cytometer. There is almost 100% of negative control A498 culture cells were viable and no apoptosis occurred after 12 hrs of incubation. However, treated cells with GI extract, Gi-ZnO NPs, and Gi-CuO NPs, the viable cell percentage reduced significantly at IC_{25}, IC_{50} and IC_{75} respectively. Furthermore, the percentage of cells at apoptotic stage increased significantly at all concentrations compared to control group.

The thesis deals with each of the above topics in detail.

CHAPTER VI – SUMMARY AND CONCLUSION

This thesis deals mainly with the preliminary qualitative, quantitative phytochemical screening, and GC-MS analysis of aqueous extracts of *Garcinia cambogia* and *Garcinia indica* leaves. Furthermore, optimization, green synthesis, and characterization of zinc oxide and copper oxide nanoparticles from *Garcinia cambogia* and *Garcinia indica* leaves and evaluation of their antioxidant as well as anticancer property in kidney cancer (A498) cell lines have been described in different chapters and the summary and conclusion for each chapter have been described below:

Chapter I

This chapter initially deals with the introductory details of cancer and its prevalence, nanotechnology and their applications. The need for choosing the medicinal plant and their advantages were explained in detail. Furthermore, the general information of ZnO and CuO NPs and their application are given in detail.

Chapter II

This section gives the literature relevant to the present study. A summary on the different types of plants used in the biosynthesis of ZnO, and CuO NPs has also been given. The literature search provides the background and guidance for the scope of present investigation.

Chapter III

This chapter briefly covers the materials required and instruments used for the study. Initially chapter deals with collection of plants, preparation of extracts, phytochemical screening and optimization and synthesis of nanoparticles (ZnO and CuO NPs). Furthermore, the instrumental techniques for characterization of synthesized nanoparticles using the techniques like UV-vis spec, FT-IR, XRD, SEM, EDAX, HR-TEM, SAED pattern, particle size distribution, and DLS of ZnO NPs and CuO NPs have been given. At the end, the chapter deals with the assay procedures of antioxidant and anticancer activities.

Chapter IV

This chapter deals with results and discussion of the entire study of GC. Starting from preliminary physicochemical and phytochemical analysis till apoptosis. Briefly the study revealed that preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, saponins and by GC-MS analysis the bioactive compounds were identified.

Furthermore, optimization of nanoparticles using aqueous leaf extract of *G. cambogia*

played a key role in synthesizing smaller nanoparticles. Characterization techniques confirmed the shape, size and morphology of synthesized nanoparticles. Biological application such as antioxidant and anticancer activity of Gc-ZnO and Gc-CuO NPs comparing with the plant extract showed dose dependent activity. Metal oxide nanoparticle and GC extract showed significant cytotoxic effect against A498 cell line without killing normal cell line. The evaluation of apoptosis by DAPI confirmed the morphological changes in both the plant extract and metal oxide nanoparticles. Finally, flow cytometer applied to quantitatively detect apoptosis rates through Annexin V-FITC/PI suggested that Gc-ZnO, Gc-CuO NPs and plant extract induced the early apoptosis rather than necrotic pathway.

Green synthesized Gc-ZnO and Gc-CuO NPs showed significant anticancer activity on A498 cancer cell line.

Chapter V

This chapter deals with results and discussion of the entire study of GI. Starting from preliminary physicochemical and phytochemical analysis till apoptosis. Briefly the study revealed that preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, saponins and by GC-MS analysis the bioactive compounds were identified.

Furthermore, optimization of nanoparticles using aqueous leaf extract of *G. indica* played a key role in synthesizing smaller nanoparticles. Characterization techniques confirmed the shape, size and morphology of synthesized nanoparticles. Biological application such as antioxidant and anticancer activity of Gi-ZnO and Gi-CuO NPs comparing with the plant extract showed dose dependent activity. Metal oxide nanoparticle and GI extract showed significant cytotoxic effect against A498 cell line without killing normal cell line. The evaluation of apoptosis by DAPI confirmed the morphological changes in both the plant extract and metal oxide nanoparticles. Finally, flow cytometer applied to quantitatively detect apoptosis rates through Annexin V-FITC/PI suggested that Gi-ZnO, Gi-CuO NPs and plant extract induced the early apoptosis rather than necrotic pathway. Green synthesized Gi-ZnO and Gi-CuO NPs showed significant anticancer activity on A498 cancer cell line.

CONCLUSION

In conclusion, Garcinia species possesses many secondary metabolites and radical scavengers, especially polyphenols which acted as capping and stabilizing agents for nanoparticle synthesis. A simple, eco-friendly and rapid approach for the synthesis of CuO and ZnO nanoparticles accomplished by using *G. cambogia* and *G. indica* leaf extracts showed good radical scavenging activity. Optimization techniques play a key role in nanoparticle synthesis and metal oxide nanoparticles showed significant anticancer activity compared to plant extracts and standard drug. Especially, CuO nanoparticles showed better anticancer activity in both plant extracts compared to ZnO NPs and plant extracts. These results highlight the use of conventional medicinal plants by hybridizing the advanced nanotechnology for the treatment of cancer which is the primary threat to modern human society.

REFERENCES

1. Liu R, Pei Q, Shou T, Zhang W, Hu J, Li W. Apoptotic effect of green synthesized gold nanoparticles from Curcuma wenyujin extract against human renal cell carcinoma A498 cells. International Journal of Nanomedicine. 2019; 14:4091.
2. Rutz J, Maxeiner S, Juengel E, Bernd A, Kippenberger S, Zöller N, Chun FK, Blaheta RA. Growth and proliferation of renal cell carcinoma cells is blocked by low curcumin concentrations combined with visible light irradiation. International Journal of Molecular Sciences. 2019 Jan; 20(6):1464.
3. Xu M, Wang Y, Zhou LN, Xu LJ, Jin ZC, Yang DR, Chen MB, Zhu J. The therapeutic value of SC66 in human renal cell carcinoma cells. Cell death & disease. 2020 May 11; 11(5):1-1.
4. Swiatek M, Jancewicz I, Kluebsoongnoen J, Zub R, Maassen A, Kubala S, Udomkit A, Siedlecki JA, Sarnowski TJ, Sarnowska E. Various forms of HIF-1α protein characterize the clear cell renal cell carcinoma cell lines. IUBMB life. 2020 Jun; 72(6):1220-32.
5. Wang X, Liu J, Xie Z, Rao J, Xu G, Huang K, Li W, Yin Z. Chlorogenic acid inhibits proliferation and induces apoptosis in A498 human kidney cancer cells *via* inactivating PI3K/Akt/mTOR signalling pathway. Journal of Pharmacy and Pharmacology. 2019 Jul; 71(7):1100-9.

6. Al-Ajmi MF, Hussain A, Alsharaeh E, Ahmed F, Amir S, Anwar MS, Siddiqui MA, Al-Khedhairy AA, Koo BH. Green synthesis of zinc oxide nanoparticles using Alstonia macrophylla leaf extract and their *in vitro* anticancer activity. Science of Advanced Materials. 2018 Mar 1; 10(3):349-55.
7. D.J. Manasa, K.R. Chandrashekar, M.A. Pavan Kumar, D. Suresh, D.J. Madhu Kumar, C.R. Ravikumar, T. Bhattacharya, H.C. Ananda Murthy, Proficient synthesis of zinc oxide nanoparticles from Tabernaemontana heyneana Wall. *via* green combustion method: Antioxidant, anti-inflammatory, antidiabetic, anticancer and photocatalytic activities, Results Chem. 3 (2021) 100178.
8. Gowdhami B, Jaabir M, Archunan G, Suganthy N. Anticancer potential of zinc oxide nanoparticles against cervical carcinoma cells synthesized *via* biogenic route using aqueous extract of Gracilaria edulis. Materials Science and Engineering: C. 2019 Oct 1; 103:109840.
9. Chandra H, Patel D, Kumari P, Jangwan JS, Yadav S. Phyto-mediated synthesis of zinc oxide nanoparticles of Berberis aristata: Characterization, antioxidant activity and antibacterial activity with special reference to urinary tract pathogens. Materials Science and Engineering: C. 2019 Sep 1; 102:212-20.
10. Klingshirn C. ZnO: material, physics and applications. ChemPhysChem. 2007 Apr 23; 8(6):782-803.
11. Wayne Richardson,H.Copper Compounds in Ullmann's Encyclopaedia of Industrial Chemistry, Wiley-VCH, Weinheim.2002. DOI: 10.1002/143560 07.a07_567.
12. Mir AH, Qamar A, Qadir I, Naqvi AH, Begum R. Accumulation and trafficking of zinc oxide nanoparticles in an invertebrate model, Bombyx mori, with insights on their effects on immuno-competent cells. Scientific reports. 2020 Jan 31; 10(1):1-4.
13. Selim YA, Azb MA, Ragab I, HM Abd El-Azim M. Green synthesis of zinc oxide nanoparticles using aqueous extract of Deverra tortuosa and their cytotoxic activities. Scientific reports. 2020 Feb 26; 10(1):1-9.
14. Caparica R, Júlio A, Araújo ME, Baby AR, Fonte P, Costa JG, Santos de Almeida T. Anticancer activity of rutin and its combination with ionic liquids on renal cells. Biomolecules. 2020 Feb; 10(2):233.
15. Geetha S, Saraswathi K, Chakrapani GD, Sivaraj C, Dhivya M, Chittibabu CV. Antioxidant and anticancer activities of pericarp of Garcinia mangostana L. Journal of Pharmacognosy and Phytochemistry. 2020; 9(4):1805-9.

16. Espirito Santo BL, Santana LF, Kato Junior WH, de Araújo FD, Bogo D, Freitas KD, Guimarães RD, Hiane PA, Pott A, Filiú WF, Arakaki Asato M. Medicinal potential of Garcinia species and their compounds. Molecules. 2020 Jan; 25(19):4513.
17. Seethapathy GS, Tadesse M, Urumarudappa SK, V Gunaga S, Vasudeva R, Malterud KE, Shaanker RU, de Boer HJ, Ravikanth G, Wangensteen H. Authentication of Garcinia fruits and food supplements using DNA barcoding and NMR spectroscopy. Scientific reports. 2018 Jul 12; 8(1):1-2.
18. Ohia SE, Opere CA, LeDay AM, Bagchi M, Bagchi D, Stohs SJ. Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). Molecular and cellular biochemistry. 2002 Sep; 238(1):89-103.
19. Abraham Z, Malik SK, Rao GE, Narayanan SL, Biju S. Collection and characterisation of Malabar tamarind [*Garcinia cambogia* (Gaertn.) Desr.]. Genetic resources and crop evolution. 2006 Mar; 53(2):401-6.
20. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestree Database: a tree reference and selection guide. Version 4. Agroforestree Database: a tree reference and selection guide. Version 4. 2009.
21. Lim, TK. Cucumis melo (Makuwa Group). Edible Medicina and Non-Medicinal Plants. Springer, Dordrecht. 2012. pp 219- 221
22. Watt G (1890). Dictionary of the economic products of India. Periodical Experts, Delhi, 3.
23. Baliga MS, Bhat HP, Pai RJ, Boloor R, Palatty PL. The chemistry and medicinal uses of the underutilized Indian fruit tree Garcinia indica Choisy (kokum): a review. Food Research International. 2011 Aug 1; 44(7):1790-9.
24. Harborne, J. B. Phytochemical Methods. Chapman and Hall Ltd, London. 1973; 2:5-11.
25. Evans, W. Trease and Evans' Pharmacognosy. Elsevier Health Sciences, New York. 2002; 21-24. 22.
26. Jaradat N, Hussen F, Al Ali A. Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata Decne. J. Mater. Environ. Sci. 2015; 6(6):1771-8.
27. Noreen H, Semmar N, Farman M, McCullagh JS. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. Asian Pacific Journal of Tropical Medicine. 2017 Aug 1; 10(8):792-801.

28. Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. Journal of King Saud University-Science. 2015 Jul 1; 27(3):224-32.
29. Mir MA, Parihar K, Tabasum U, Kumari E. Estimation of alkaloid, saponin and flavonoid, content in various extracts of Crocus sativa. Journal of Medicinal Plants Studies. 2016; 4(5):171-4.
30. Prabhadevi V, Sahaya SS, Johnson M, Venkatramani B, Janakiraman N. Phytochemical studies on Allamanda cathartica L. using GC-MS. Asian Pacific Journal of Tropical Biomedicine. 2012 Feb 1; 2(2):S550-4.
31. Singh, D., Rathod, V., Ninganagouda, S., Hiremath,J., Singh, A. K., Mathew, J. Optimization and characterization of silver nanoparticle by endophytic fungi Penicillium sp. isolated from *Curcuma longa* (turmeric) and application studies against MDR *E. coli* and *S. aureus*. Bioinorganic Chemistry and Applications. 2014 Jan 1; 2014.
32. Zare E, Pourseyedi S, Khatami M, Darezereshki E. Simple biosynthesis of zinc oxide nanoparticles using nature's source, and it's *in vitro* bio-activity. Journal of Molecular Structure. 2017 Oct 15; 1146:96-103.
33. Nm JO, Monika S, Alok S, Alok M, Shruti R. *In vitro* and *in vivo* models for antioxidant activity evaluation: A review.International journal of pharmaceutical sciences and research.2010; 1(1):1-1.
34. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 1995 Jan 1; 28(1):25-30.
35. Benzie, I. F., Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Analytical Biochemistry. 1996 Jul 15; 239(1): 70-76.
36. Prieto, P., Pineda, M., Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry. 1999 May 1; 269(2): 337-341.
37. Sudha, K. G., Ali, S., Karunakaran, G., Kowsalya, M., Kolesnikov, E., Rajeshkumar, M. P. Eco-friendly synthesis of ZnO nanorods using *Cycas pschannae* plant extract with excellent photocatalytic, antioxidant, and anticancer

nanomedicine for lung cancer treatment. Applied Organometallic Chemistry. 2020 Apr; 34(4): e5511

38. J. Todorova, L.I. Lazarov, M. Petrova, A. Tzintzarov, I. Ugrinova, The antitumor activity of cannabidiol on lung cancer cell lines A549 and H1299: the role of apoptosis, Biotechnol. Equip. 35 (2021) 873–879.
39. Boroumand Moghaddam A, Moniri M, Azizi S, Abdul Rahim R, Bin Ariff A, Navaderi M, Mohamad R. Eco-friendly formulated zinc oxide nanoparticles: induction of cell cycle arrest and apoptosis in the MCF-7 cancer cell line. Genes. 2017 Oct; 8(10):281.
40. Murali M, Mahendra C, Rajashekar N, Sudarshana MS, Raveesha KA, Amruthesh KN. Antibacterial and antioxidant properties of biosynthesized zinc oxide nanoparticles from *Ceropegia candelabrum* L.–an endemic species. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2017 May 15; 179:104-9.

LIST OF PUBLICATIONS

1. **Varsha Jayakar,** Vinayak Lokapur, Manjula Shantaram. "*In vitro* antioxidant and selective cytotoxicity of *Garcinia cambogia* and *Garcinia indica* leaf extracts on human kidney cancer cell line". International Journal of Research in Pharmaceutical Sciences. 2021; Vol. 12(3): 1718-1728 (ISSN: 0975-7538).
2. **Varsha Jayakar**, Vinayak Lokapur, Nityasree B. R., Raju Krishna Chalannavar, Lyned D. Lasrado, Manjula Shantaram*. "Optimization and green synthesis of zinc oxide nanoparticle using *Garcinia cambogia* leaf and evaluation of their antioxidant and anticancer property in kidney cancer (A498) cell lines". Biomedicine, 2020; 41(2): 206-222. ISSN: 0970 2067.
3. **Varsha Jayakar,** Vinayak Lokapur, Manjula Shantaram*. "Identification of the volatile bioactive compounds by GC-MS analysis from the leaf extracts of *Garcinia cambogia* and *Garcinia indica*". Medicinal Plants- International Journal of Phytomedicines and Related Industries, 2020; Vol.12 (4). 580-590. Print ISSN: 0975-4261. doi: 10.5958/0975- 6892.2020.00070.2

BOOK CHAPTER PUBLICATIONS

1. **Varsha Jayakar**, Vinayak Lokapur, Manjula Shantaram*. (2020) "The Role of Nanotechnology in Food Processing and Food Packaging Industries" Published Book Chapter in Nanotechnology in Food Industry: Applications, Challenges, and Opportunities. Daiichi Books.
2. **Varsha Jayakar,** Vinayak Lokapur Manjula Shantaram*. (2020) " The Role of Nanotechnology in Functional Food and its Application: Food Safety, Regulation and Challenges" accepted the Book chapter in Bentham Science Publications.

POSTER PRESENTATION

1. Poster presentation on "Evaluation of free radical scavenging in different leaf extracts of *Garcinia cambogia* and *Garcinia indica*" during the 40th Annual Conference of Indian Association of Biomedical Scientists (IABMS) on "Biomedical research and innovation to enhance Quality of life" held from 31st Oct - 2nd November 2019 at Chettinad Hospital and Research Institute (CHRI) Kelambakkam.
2. Poster presentation on "A review on stem cells from umbilical cord blood" during 39th International conference on Current concepts on the role of Indian medicine and

phytoceuticals in maintenance of health held from 15th to 17th November at Jnana Kaveri PG Centre, Chikka Aluvara, Mangalore University.

3. Poster presentation on "A perception of radiation effects among the individuals of Jnana Kaveri campus - a survey" during the International Conference on Radiation Biology under the auspices of Indian Society of Radiation Biology theme being "Advances in Radiation Biology-Radiotherapy and Radiation Countermeasures" held from 4th to 6th October 2018 at K. S. Hegde Medical Academy, Deralakatte, Mangaluru

WORKSHOP AND CONFERENCES ATTENDED

- Participated in the DBT Ministry of Science and Technology, New Delhi Sponsored Ten Days National workshop on "Plant Microbial Interaction for the production of the Therapeutic Compounds" held from 17th to 26th October 2019 at Kuvempu University.
- Participated in the Dr. Sarada Subramaniam Preconference workshop on Handling of laboratory animals & Zebra fish in biological research" in the 40th Annual Conference of Indian Association of Biomedical Scientists (IABMS) on "Biomedical research and innovation to enhance quality of life" held from 31st Oct 2019 at Chettinad Hospital and Research Institute (CHRI) Kelambakkam.
- Participated in the Interactive Workshop on "Arecanut and Human Health" held during 24th to 25th July 2018 at Central Plantation Crops Research Institute, Kasaragod, Kerala.
- Participated in Two Day Lecture Series in Molecular Genetics held on 24th and 25th August 2018 at K. S. Hegde Medical Academy, Deralakatte, Mangaluru in association with Karnataka Science and Technology Academy (KSTA).
- Participated in workshop on "Basics of cell culture and application in drug discovery" during the International Conference on "Current concepts on the role of Indian medicine and phytoceuticals in maintenance of health" under the auspices of 39th Annual Summit of Indian Association of Biomedical Scientists (IABMS) held from 15th to 17th November 2018 at Jnana Kaveri Post Graduate Centre of Mangalore University Chikka Aluvara, Kodagu.
- Participated in workshop on "DNA Damage Studies" at International Conference on Radiation Biology under the auspices of Indian Society of Radiation Biology theme being "Advances in Radiation Biology-Radiotherapy and Radiation Countermeasures" held on 4th October 2018 at K. S. Hegde Medical Academy, Deralakatte, Mangaluru.